UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

APR 16 2018

*****CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION in CBI APPENDICES*****

MEMORANDUM

SUBJECT: Bacteriophages active against *Xylella fastidiosa.*

TO: Alexandra Boukedes, Risk Manager
Microbial Pesticides Branch,
Biopesticides and Pollution Prevention Division (7511P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Risk Assessment Branch,
Biopesticides and Pollution Prevention Division (7511P)

THROUGH: John L. Kough, Ph.D., Senior Scientist
Risk Assessment Branch,
Biopesticides and Pollution Prevention Division (7511P)

ACTION REQUESTED: Review submitted studies for product characterization of Bacteriophages active against *Xylella fastidiosa* and studies to support a new end-use product and a food tolerance exemption.

CONCLUSIONS:
Product Identity and Characterization – **SUPPLEMENTAL but upgradeable**.
Waiver request for Cell Culture – **ACCEPTABLE**.
Waiver requests for Inhalation Toxicity, Oral, Pulmonary and Injection Toxicity/Pathogenicity – **ACCEPTABLE**.
Acute Oral and Dermal Toxicity – **ACCEPTABLE** – EPA Toxicity Category IV.
Dermal and Eye Irritation – **ACCEPTABLE** – EPA Toxicity Category IV.
Skin Sensitization – **SUPPLEMENTAL** – likely not a dermal sensitizer – not a required study.
Food Tolerance Exemption Petition – **ACCEPTABLE**.

DATA REVIEW RECORD:

Active Ingredients:	Bacteriophages active against *Xylella fastidiosa.*
Product Names:	OPC-721; OPC-821.
Company Name:	Otsuka Pharmaceutical Co., LTD.
EPA Reg. Nos.:	92918-R; 92918-E; 7F8562.
Chemical Numbers:	116404.
Decision Numbers:	527226; 527227; 527288.
DP Barcodes:	444193; 444194; 444202.
MRID Nos.:	501593-01; 501593-02; 501593-04; 501593-05; 501593-06; 501593-07; 501593-08; 503233-01; 503489-01; 503641-01; 505501-01.

Note: MRID 505501-01 was submitted requesting a waiver for Hypersensitivity Incidents under 885.3400. The guideline 885.3400 is not a testing requirement of registration but rather an ongoing duty to report any incidents of Hypersensitivity related to pesticide use "including immediate type and delayed-type reactions of humans or domestic animals, [that] occur during the testing or production of the TGAI, MP, or EP, or are otherwise known to the applicant must be reported if they occur" under FIFRA 6(a)(2) adverse effects reporting procedures. For more information, see: https://www.epa.gov/pesticide-incidents/incident-reporting-pesticide-manufacturers-registrants

DATA EVALUATION RECORD

Review by: Joel V. Gagliardi, Ph.D.
Secondary review by: John L. Kough, Ph.D.

Study Types	Product Identity (885.1100); Manufacturing Process (885.1200); Culture Collection Deposition (885.1250); Discussion of Formation of Unintentional Ingredients (885.1300); Analysis of Samples (885.1400); Certification of Limits (885.1500); Physical and Chemical Characteristics (830.6302 - 830.7300).
MRID Nos.	501593-01; 501593-02; 503233-01; 503489-01.
Test Material	OPC-721 and OPC-821 containing $1x10^{10}$ PFU/mL Bacteriophages active against *Xylella fastidiosa.*
Study Nos.	None; 43870; None; None.
Sponsor	Otsuka Pharmaceutical Company Ltd., 2-9 Kanda-Tsukasamachi; Chiyoda-ku, Tokyo, 101-8535, Japan.
Testing Facilities	Technology Sciences Group Inc., 712 Fifth Street, Suite A, Davis, California 95618; Product Safety Labs; Otsuka Pharmaceutical Company Ltd., 2-9 Kanda-Tsukasamachi; Chiyoda-ku, Tokyo, 101-8535, Japan.
Titles of Reports	Product Chemistry for OPC-721; Xylophage_V1: Physical and Chemical Characteristics; Supplemental Product Chemistry for OPC-721; Supplemental Product Chemistry References for Bacteriophages active against *Xylella fastidiosa.*
Authors	Jacob S. Moore; Catherine Wo, Ph.D.; Jacob S. Moore; Jacob S. Moore.
Studies Completed	March 10, 2017; December 7, 2016; July 11, 2017; August 11, 2017.
Study Summaries	Bacteriophage are obligate intracellular parasites requiring a bacterial host for replication and exist anywhere a suitable host may be found, or remain dormant in whatever environment they are transported to. Estimates are that bacteriophages are the most abundant organism in nature. These bacteriophages are in the order Caudovirales families Podoviridae or Siphoviridae. The Podoviridae have isometric heads and short, stubby tails while the Siphoviridae have isometric heads and non-contractile tails. The initial bacteriophages were isolated from plants, soils, or from other environmental sources. Phages have two basic modes of existence, as temperate or lytic. Temperate phage may integrate into the host genome and replicate as part of the cell, while lytic phage, which comprise those described here, do not undergo integration but rather obligately replicate then lyse the host cell via a cassette of lysis genes that includes holins and pinholins (that may be controlled by antiholins), endolysins, and spanins. The pest host range is *Xylella* and *Xanthomonas* spp. or any Gram negative prokaryote that has a type IV pilus. At least two phages were able to lyse each *Xylella* strain tested though only the Genus *Pradovirus* was able to lyse some of the tested *Xanthomonas* strains. The registrant has proposed criteria used to add phages to this product: 1) ruling out lysogenic phage in bacterial culture and from genetic analysis, and 2) confirming a lytic cycle in a bacterial culture and from the presence of lytic genes from a whole genome sequence.
Classification	**SUPPLEMENTAL but upgradeable when** – 1) Storage stability and corrosion characteristics data are submitted; and 2) analysis of the genome sequence for each phage is provided where any contiguous protein sequence similarity ≥35% to naturally occurring mammalian toxins is identified.
Good Laboratory Practice	Signed and dated GLP statements were included: MRID 501593-02 was conducted according to 40 CFR part 60 except characterization of the test substance was the responsibility of the sponsor; other MRIDs are not studies.

I. PRODUCT IDENTITY:

II. Active Ingredient Name: Bacteriophages active against *Xylella fastidiosa.*

Trade Names: OPC-721; OPC-821.

Name and Address of Applicant: Otsuka Pharmaceutical Company Ltd., 2-9 Kanda-Tsukasamachi; Chiyoda-ku, Tokyo, 101-8535, Japan.

Name and Address of Manufacturing Plant: Texas A&M University Center for Phage Technology; 300 Olsen Blvd., MS 2128 Rm 314; College Station, Texas 77843.

Common Names: Paz, Prado, Salvo, Sano.

Deposition in a culture collection: see CSF.

Regulatory Status: No yet registered or in use anywhere.

A. INTENTIONALLY ADDED INERT INGREDIENTS: see CSF.

B. CHARACTERIZATION OF THE MPCA:

i) Taxonomic designation: These bacteriophages are in the order Caudovirales and currently assigned to the following:

Paz	Family Podoviridae	Genbank: KF626666
Prado	Family Podoviridae	Genbank: KF626667
Salvo	Family Siphoviridae	Genbank: KF626668
Sano	Family Siphoviridae	Genbank: KF626665

The International Committee on Taxonomy of Viruses (ICTV) has proposed in 2016.079a-dB to create a new genus as *Pradovirus* for Prado in the family Podoviridae, subfamily Autographivirinae. The Podoviridae phage have isometric heads and short, stubby tails while the Siphoviridae phage have isometric heads and non-contractile tails, per micrographs (Fig. 1, MRID 503233-01).

ii) Alternatives/synonyms/superseded names associated with the microorganism: XylPhage; Sano = Xfas103; Salvo = Xfas106; Prado = Xfas303; Paz = Xfas304.
iii) Strain origins: Plants such as rice that harbor *Xanthomonas* spp., from other infected plants and environmental sources.
iv) Natural occurrence of the microorganism: The initial bacteriophages were isolated from plants (grapevines, weeds, water) soil or from other sources (Ahern et al. 2014). Bacteriophage are obligate intracellular parasites requiring a bacterial host for replication and exist anywhere a suitable host may be found, or remain dormant in whatever environment they are transported to. Estimates are that bacteriophages are the most abundant organism in nature (Clokie et al. 2011).
v) Mode of Action: Phages have two basic modes of existence, as temperate or lytic. Temperate phage may integrate into the host genome and replicate as part of the cell, while under certain conditions these phages may excise, undergo replication and exit from the cell, sometimes via cell lysis. Lytic phage, which comprise those described here (Ahern et al. 2012), do not undergo integration but rather obligately replicate then lyse the host cell via a cassette of lysis genes that includes holins and pinholins (that may be controlled by antiholins), endolysins, and spanins.
vi) Pest host range: *Xylella* spp., *Xanthomonas* spp. and any other prokaryote that has a compatible type IV pilus. At least two phages were able to lyse each of the *Xylella* strains tested though only the Genus *Pradovirus* was able to lyse some of the tested *Xanthomonas* strains. Tested bacteria that were not lysed are listed in MRID 503233-01.
vii) Life cycle: Obligate intracellular replication in infected prokaryotes, then lysis of the cell.
viii) Growth temperature range: This is not directly applicable since bacteriophages are dependent on the host cell to replicate at an optimal 29±1°C in Tryptone Yeast-Extract Potassium (TYK) broth.
ix) History of use: These bacteriophages are newly isolated and described (Ahern et al. 2014).

II. MANUFACTURING PROCESS: see the CBI Appendix.

III. CULTURE COLLECTION DEPOSITION: see the CBI appendix.

IV. DISCUSSION OF FORMATION OF UNINTENTIONAL INGREDIENTS: see the CBI appendix.

V. ANALYSIS OF SAMPLES: presented in Table 2.

Table 2. Analysis of three batches of Bacteriophages active against *Xylella fastidiosa* – OPC-721 and OPC-821.

Batch No.	PFU/mL	Sterility
CPTV1-004	1.9×10^{10}	PASS
CPTV1-001	2.9×10^{10}	PASS
CPTV1-003	1.6×10^{10}	PASS
STANDARD	$>1.0 \times 10^{10}$	STERILE
METHOD	SOP CPT-04-006	SOPs PT-04-007 for total aerobic bacteria, CPT-04-007 for coliforms and CPT-04-007 for total fungi.

VI. CERTIFICATION OF LIMITS: see the CBI appendix. Inert ingredients have existing food tolerance exemptions at 40 CFR 180.910 (inert ingredients used pre- and post-harvest) and 180.920 (inert ingredients used pre-harvest).

VII. SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES: presented in Table 4.

TABLE 4. Description of Chemical and Physical Properties for OPC-721 and OPC-821 containing Bacteriophages active against *Xylella fastidiosa*.

Guideline	Property	Result	Method/Reference
830.6302	Color	Clear colorless	Visual inspection
830.6303	Physical State	Clear liquid	Visual inspection
830.6304	Odor	None	Olfactory inspection
830.6313	Stability	At least 14 days; 42°C	Assumed viability checks
830.6317	Storage Stability	Ongoing – later submission	
830.6319	Miscibility	Not required	Not required
830.6320	Corrosion characteristics	Ongoing – later submission	
830.7000	pH	6.53	pH meter
830.7100	Viscosity	1.0 cS (20°C); 0.7 cS (40°C)	Capillary viscometer
830.7300	Density/relative density	0.982 g/mL (8.20 lbs/gallon) 20°C	Gravity bottle

DEFICIENCIES: Storage stability and corrosion characteristics data have not been submitted.

REFERENCES:

Ahern, S.J., M. Das, T.S. Bhowmick, R. Young, and C.F. Gonzalez. 2012. Characterization of Novel Virulent Broad-Host-Range Phages of *Xanthomonas* and *Xylella fastidiosa*. Journal of Bacteriology 196(2):459-471.

Clokie, M.R.J.,A.D. Millard,A.V. Letarov,and S. Heaphy. 2011. Phages in nature. Bacteriophage 1(1):31-45.

Negi, S.S., C.H. Schein, G.S. Ladics, H. Mirsky, P. Chang, J.-B. Rascle, J. Kough, L. Sterck, S. Papineni, J.M. Jez, L.P. Mouriès, and W. Braun. 2017. Functional classification of protein toxins as a basis for bioinformatics screening. Nature Scientific Reports 7(13940):1-11.

FIFRA CBI APPENDIX

Manufacturing process information may be entitled to confidential treatment

Manufacturing process information may be entitled to confidential treatment

DATA EVALUATION RECORD

Review by: Joel V. Gagliardi, Ph.D.
Secondary review by: John L. Kough, Ph.D.

Study Type	Waiver request for: Cell Culture (OCSPP 885.3500).
MRID No.	505501-01.
Test Material	Bacteriophages active against *Xylella fastidiosa.*
Study No.	None.
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Technology Sciences Group; 1150 18th St., NW, Suite 1000; Washington, DC 20036.
Title of Report	Revised Response to Tier 1 Microbial Pesticide Data Requirements for Bacteriophage active against *Xylella fastidiosa.*
Author	Leslie E. Patton, Ph.D.
Study Completed	December 26, 2017.
Study Summary	The purpose of the Cell Culture guideline, specific for any pesticidal viruses, is to test in mammalian cell lines for potential infectivity and pathogenesis of virus active ingredients. As discussed above, bacteriophage as a group are obligate intracellular parasites of prokaryotes and have no ability to replicate in mammalian cells or cell lines. In particular these bacteriophages are specific to the prokaryotic type IV pilus of Gram negative bacteria species and while they cannot infect a cell lacking this phenotype, product characterization selects for only lytic bacteriophages for use in these products, confirmed in culture and with whole genome sequencing and analysis. Each isolate is analyzed to confirm they are lytic and not able to integrate into a host genome and to rule out carriage of extraneous DNA between cells.
Classification	**ACCEPTABLE**.
Good Laboratory Practice	Signed and dated GLP statement was provided; this MRID was not a study so 40 CFR 160 does not apply.

DATA EVALUATION RECORD

Review by: Joel V. Gagliardi, Ph.D.
Secondary review by: John L. Kough, Ph.D.

Study Types	Waiver requests for: Acute Inhalation Toxicity (OCSPP 870.1300); Acute Oral (OCSPP 885.3050), Pulmonary (OCSPP 885.3150), and Injection (OCSPP 885.3200) Toxicity and Pathogenicity.
MRID No.	503641-01.
Test Material	Bacteriophages active against *Xylella fastidiosa*.
Study No.	None
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Technology Sciences Group; 1150 18th St., NW, Suite 1000; Washington, DC 20036.
Title of Report	Supplemental Response to Tier 1 Microbial Pesticide Data Requirements for Bacteriophage active against *Xylella fastidiosa*.
Author	Leslie E. Patton, Ph.D.
Study Completed	September 13, 2017.
Study Summary	The purpose of the Toxicity and Pathogenicity series 885.xxxx guidelines, specific for microbes, is to test in animals at a maximum hazard dose for potential infectivity and pathogenesis of an active ingredient. As discussed above, bacteriophage as a group are obligate intracellular parasites of prokaryotes and have no ability to replicate in mammalian cells. These bacteriophages are specific to the prokaryotic type IV pilus of certain Gram negative bacterial species and cannot infect a cell lacking this phenotype. The utility of testing for infectivity in animals and any overt toxicity or pathogenicity is moot. Bacteriophages are the most plentiful biological organism known, present in soils and water (salt and fresh) and in or on foods of all types. Only rarely are bacteriophages associated with negative effects, most commonly involving carriage of DNA between bacterial cells by temperate phage. Only lytic bacteriophages are utilized in these products, confirmed in culture and with whole genome sequencing and analysis. Each isolate is analyzed to confirm they are lytic and not able to integrate into a host genome and to rule out carriage of extraneous DNA between cells, minimizing any concern of off-target effects.
Classification	**ACCEPTABLE**.
Good Laboratory Practice	Signed and dated GLP statement was provided; this MRID was not a study so 40 CFR 160 does not apply.

DATA EVALUATION RECORD

Review by: Donna L. Fefee, D.V.M.; Summitec Corporation.
EPA review by: Joel V. Gagliardi, Ph.D.

Study Type	Acute Oral Toxicity - Rat (OCSPP 870.1100).
MRID No.	501593-04.
Test Material	OPC-721 containing $1.9x10^{10}$ PFU/mL Bacteriophages active against *Xylella fastidiosa*.
Study No.	20105084.
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Charles River Laboratories, Inc., 640 N. Elizabeth Street, Spencerville, Ohio.
Titles of Report	Acute oral toxicity study of OPC-721 in rats.
Author	K. Landin.
Study Completed	November 18, 2016.
Study Summary	In an acute oral toxicity study, a group of five fasted female 8-week-old Sprague Dawley Crl:CD(SD) rats were given a single oral gavage dose of 5,000 mg/Kg bw (5 mL/Kg bw dosing volume) of undiluted OPC-721. Dosing was on Day 0, and the animals were observed for up to 14 days. Based on the results of this study, OPC-721 showed no toxicity in the female rat after exposure to a single oral dose of 5,000 mg/Kg bw. There were no deaths, abnormal clinical signs, or abnormal gross necropsy findings, and all of the animals gained weight during both weeks of the study.
Classification	**ACCEPTABLE** – Oral toxic or lethal dose Females >5,000 mg/Kg bodyweight – EPA Toxicity Category IV.
Good Laboratory Practice	Signed and dated GLP statement was provided; the study is compliant with 40 CFR part 160, except the Sponsor was responsible for the expiration date and stability and/or solubility testing of the supplied test substance.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test material:**	OPC-721.
	Description:	Transparent, colorless liquid.
	Lot/Batch #:	CPTV1-004.
	Purity:	$1.9x10^{10}$ PFU/mL.
	CAS #:	Not applicable.
	Storage conditions:	Stored in a refrigerator (5°C), protected from light.
	Microbiology:	$1.9x10^{10}$ PFU/mL.

2. **Sample preparation:** The test material was administered as received.

3. **Controls:** None.

4.	**Test animals:**		
	Species:	Albino rat.	
	Strain/Source:	Sprague Dawley Crl:CD(SD); Charles River Laboratories, Raleigh, North Carolina.	
	Animals Assigned:	5 females (nulliparous and non-pregnant).	
	Age/weight at dosing:	Approximately 8 weeks / females: 164-195 g.	
	Housing:	Individually in polycarbonate cages containing appropriate bedding.	
	Diet:	PMI Nutrition International Certified Rodent Chow No. 5CR4; *ad libitum*.	
	Water:	Municipal water; *ad libitum*.	
	Environmental conditions:	**Temperature:**	21-22°C
		Humidity:	46-57%
		Air changes:	>10/hour
		Photoperiod:	12 hr dark/12 hr light
	Acclimation period:	6 days	

B. STUDY DESIGN AND METHODS:

1. **In life dates:** Start: September 12, 2016; End: September 26, 2017.

2. **Preliminary challenge assay:** No preliminary work was conducted.

3. **Animal assignment and treatment:** All animals were assigned to a single test group. After overnight fasting, undiluted test material was dosed at 5,000 mg/Kg bodyweight by gavage.

4. **Clinical observations and bodyweight:** Bodyweight was recorded prior to dosing, and on days 7 and 14. The test animals were observed for mortality and clinical signs of toxicity at least twice on the day of dosing and once daily thereafter for 14 days.

5. **Feed consumption:** Food consumption was not reported.

6. **Necropsy and organ weight determination:** All animals were euthanized by carbon dioxide inhalation and necropsied on day 14 which included evaluation of the following: the carcass and musculoskeletal system; all external surfaces and orifices; the cranial cavity and external surfaces of the brain; and the thoracic, abdominal, and pelvic cavities

with their associated organs and tissues. No tissues were retained. The study author did not state whether the test animals were fasted overnight prior to sacrifice.

7. **Microbial enumeration:** not reported.
8. **Sensitivity of detection:** not reported.
9. **Raw Data:** not included.
10. **Statistics:** not applicable.

II. RESULTS

A. MORTALITY: All animals survived the study. The Oral toxic or lethal dose Females Females >5,000 mg/Kg bodyweight.
B. CLINICAL OBSERVATIONS: There were no abnormal clinical signs noted.
C. BODYWEIGHT: All animals gained weight normally throughout the study.
D. FEED CONSUMPTION: Feed consumption was not reported.
E. NECROPSY: No observable abnormalities were found at necropsy.
F. ORGAN WEIGHTS: Organ weights were not reported.
G. MICROBIAL ENUMERATION: not reported.

III. CONCLUSIONS

STUDY AUTHOR'S CONCLUSIONS: Under the conditions of this study, the acute oral LD_{50} of OPC-721 was determined to be greater than 5,000 mg/Kg in the female rat. The test substance would be classified in EPA Toxicity Category IV for labelling.

DEFICIENCIES: None.

DATA EVALUATION RECORD

Review by: Donna L. Fefee, D.V.M.; Summitec Corporation.
EPA review by: Joel V. Gagliardi, Ph.D.

Study Type	Acute Dermal Toxicity – Rat (OCSPP 870.1200).
MRID No.	501593-05.
Test Material	OPC-721 containing $1.9x10^{10}$ PFU/mL Bacteriophages active against *Xylella fastidiosa.*
Study No.	20105085.
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Charles River Laboratories, Inc., 640 N. Elizabeth Street, Spencerville, Ohio.
Title of Report	Acute dermal toxicity study of OPC-721 in rats.
Author	K. Landin.
Study Completed	November 18, 2016.
Study Summary	In an acute dermal toxicity study, five male and five female 8-week-old Sprague Dawley Crl:CD(SD) rats were dermally exposed to undiluted OPC-721 applied to clipped application sites comprising approximately 10% of the body surface area for 24 hours at a dose level of 5,000 mg/Kg bw (5 mL/Kg bw dosing volume). The animals were treated on day 0 and observed for 14 days. In this study, OPC-721 showed essentially no toxicity in the rat after exposure to a single dose of 5,000 mg/Kg bw by the dermal route. There were no deaths or treatment-related gross necropsy findings, and all of the animals gained weight during both weeks of the study. Two males had red fur staining of the muzzle and/or wet urogenital fur on Day 0 post-dosing, with recovery by Day 2.
Classification	**ACCEPTABLE** – Dermal toxic or lethal dose Combined >5,000 mg/Kg bodyweight – EPA Toxicity Category IV.
Good Laboratory Practice	Signed and dated GLP statement was provided; the study is compliant with 40 CFR part 160, except the Sponsor was responsible for the expiration date and stability and/or solubility testing of the supplied test substance.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test material:**	OPC-721.
	Description:	Transparent, colorless liquid.
	Lot/Batch #:	CPTV1-004.
	Purity:	$1.9x10^{10}$ PFU/mL.
	CAS #:	Not applicable.
	Storage conditions:	Stored in a refrigerator (5°C), protected from light.
	Microbiology:	$1.9x10^{10}$ PFU/mL.

2. **Sample preparation:** The test material was administered as received.

3. **Controls:** None.

4.	**Test animals:**		
	Species:	Albino rat.	
	Strain/Source:	Sprague Dawley Crl:CD(SD); Charles River Laboratories, Raleigh, North Carolina.	
	Animals Assigned:	5/sex (females nulliparous and non-pregnant).	
	Age/weight at dosing:	Approximately 8 weeks / males 215-233 g; females: 170-192 g.	
	Housing:	Individually in polycarbonate cages containing appropriate bedding.	
	Diet:	PMI Nutrition International Certified Rodent Chow No. 5CR4; *ad libitum.*	
	Water:	Municipal water; *ad libitum.*	
	Environmental conditions:	**Temperature:**	20-26°C.
		Humidity:	30-70%.
		Air changes:	>10/hour.
		Photoperiod:	12 hr dark/12 hr light.
	Acclimation period:	6 days.	

B. STUDY DESIGN AND METHODS:

1. **In life dates:** Start: September 12, 2016; End: September 26, 2016.

2. **Preliminary challenge assay:** None.

3. **Animal Assignment and Treatment:** All animals were assigned to a single treatment group. On Day 0, the test animals were dermally exposed for 24 hours to undiluted OPC-21 at a dose level of 5,000 mg/Kg bw. The test material was applied to a previously clipped area of skin on the dorsal surface (approximately 10% of the total body surface area) then covered with 4-ply gauze, plastic wrap and an elastic wrap around the entire trunk of the animal held in place with tape. At the end of the exposure period, the application site was washed with a gauze patch moistened in distilled water.

4. **Clinical Observations and Body Weight:** Clinical observations were performed on all animals at least twice daily for 14 days. Individual observations were performed on the skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, somatomotor activity, and behavior. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Body weights were recorded on the

day of treatment (Day 0) and weekly thereafter (Days 7 and 14).
5. Feed consumption: Feed consumption was not reported.
6. Necropsy: All animals were euthanized by carbon dioxide inhalation and necropsied on day 14 which included evaluation of the following: the carcass and musculoskeletal system; all external surfaces and orifices; the cranial cavity and external surfaces of the brain; and the thoracic, abdominal, and pelvic cavities with their associated organs and tissues No tissues were retained. The study author did not state whether the test animals were fasted overnight prior to sacrifice.
7. Statistics: Not applicable.

II. RESULTS
A. MORTALITY: All rats survived the study. **Combined dermal toxic or lethal dose >5,000 mg/Kg bodyweight.**
B. CLINICAL OBSERVATIONS: Two males had red fur staining of the muzzle and/or wet urogenital fur on Day 0 post-dosing, with recovery by Day 2. Three males and two females had skin scabs and/or red skin on the dorsal cervical, scapular, or interscapular areas, all of which were present prior to treatment and thus considered incidental findings (non-treatment-related). There were no observations of erythema, edema, and/or other signs of dermal irritation noted on any application site at any time point after treatment.
C. BODYWEIGHT: All animals gained weight normally throughout the study.
D. FEED CONSUMPTION: Feed consumption was not reported.
E. NECROPSY: Gross findings were limited to skin scabs on 2/5 males that correlated with observations noted during the in-life phase of the study.

III. CONCLUSION
STUDY AUTHOR'S CONCLUSIONS: Under the conditions of this test, OPC-721 was considered non-toxic, and the acute dermal LD_{50} of OPC-721 was estimated to be greater than 5,000 mg/Kg in the rat. The test substance would be assigned to EPA Toxicity Category IV for labeling.

DEFICIENCIES: Three males and two females had skin scabs and/or red skin on the dorsal cervical, scapular, or interscapular areas present prior to treatment and thus considered incidental findings and should have been disqualified from this study, though this deficiency did not affect the study conclusions in this case.

DATA EVALUATION RECORD

Review by: Donna L. Fefee, D.V.M., Summitec Corporation.
EPA review by: Joel V. Gagliardi, Ph.D.

Study Type	Primary Eye Irritation – Rabbit (OCSPP 870.2400).
MRID No.	501593-06.
Test Material	OPC-721 containing $1.9x10^{10}$ PFU/mL Bacteriophages active against *Xylella fastidiosa*.
Study No.	20105086.
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Charles River Laboratories, Inc., 640 N. Elizabeth Street, Spencerville, Ohio.
Title of Report	Primary ocular irritation study of OPC-721 in rabbits.
Author	K. Landis.
Study Completed	November 18, 2016.
Study Summary	In an acute eye irritation study, a 0.1 mL volume of undiluted OPC-721 was instilled into the conjunctival sac of the anesthetized right eye of three 13-week-old female New Zealand White rabbits, and the upper and lower lids were held shut for approximately one second, while the anesthetized but otherwise untreated left eye of each animal served as control. Eyes were scored for ocular irritation according to the Draize method at 1, 24, 48, and 72 hours after instillation with fluorescein staining done at 24 hours and as needed thereafter. The Maximum Mean Total Score was 0.00, which classifies OPC-721 as non-irritating to the eye according to the scoring system of Kay and Calandra.
Classification	**ACCEPTABLE** – not irritating to the eye – EPA Toxicity Category IV.
Good Laboratory Practice	Signed and dated GLP statement was provided; the study is compliant with 40 CFR part 160, except the Sponsor was responsible for the expiration date and stability and/or solubility testing of the supplied test substance.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test material:**	OPC-721.
	Description:	Transparent, colorless liquid.
	Lot/Batch #:	CPTV1-004.
	Purity:	$1.9x10^{10}$ PFU/mL.
	CAS #:	Not applicable.
	Storage conditions:	Stored in a refrigerator (5°C), protected from light.
	Microbiology:	$1.9x10^{10}$ PFU/mL.

2. **Sample preparation:** The test material was used as received, undiluted.

3. **Controls:** The untreated contralateral eye served as control.

4.	**Test animals:**		
	Species:	Rabbit.	
	Strain:	New Zealand White; Charles River Laboratories, St. Constant, Quebec, Canada.	
	Age/weight at dosing:	3 females (nulliparous and non-pregnant).	
	Source:	Approximately 13 weeks / females: 2.6 Kg.	
	Housing:	Individually in stainless-steel cages with an enrichment device (stainless-steel rattle).	
	Diet:	PMI Nutrition International Certified Rabbit Chow No. 5322, *ad libitum*. Timothy Hay Cube 3x/week.	
	Water:	Municipal water; *ad libitum*.	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	21°C 50-57% >10/hour 12 hr dark/12 hr light
	Acclimation period:	7 days	

B. STUDY DESIGN AND METHODS:

1. **In life dates**: Start: September 13, 2016; End: September 19, 2016.

2. **Animal assignment and treatment:** The animals were assigned to a single (treated) group. Treatment was sequential; following the absence of severe effects in an initial treated animal, the next two animals were treated concurrently. On Day 0 (prior to dosing), both eyes of each animal were examined macroscopically for ocular irritation with the aid of an auxiliary light source, including the usage of fluorescein staining. Only animals free of pre-existing ocular irritation, corneal injury, and/or fluorescein dye retention were used in the study. A minimum of one hour after the preliminary ocular examination, the undiluted test substance was instilled into the conjunctival sac of the right eye of each animal and the eyelids were gently held together for approximately one second. The contralateral eye remained untreated to serve as a control.

3. **Other conditions:** The study provided balanced pre-emptive pain control. Approximately one hour prior to treatment, buprenorphine (0.01 mg/Kg bw) was administered by subcutaneous injection to provide a therapeutic level of systemic analgesia. Approximately five minutes prior to dosing, one drop of a topical ocular anesthetic (0.5%

tetracaine hydrochloride; lot no. 237572F; expiration date: Nov 2016), was applied to each eye. In order to avoid possible interference with the study, a topical anesthetic without preservatives was used. Approximately 8 hours after dosing, buprenorphine (0.01 mg/Kg bw) was given via subcutaneous injection in order to provide a continued therapeutic level of systemic analgesia. After the initial post-treatment injection, buprenorphine was to be given approximately every 12 hours, until ocular lesions were resolved and no clinical signs of pain or distress were present.

4. Clinical observations and body weights: Clinical signs of toxicity, if present, were recorded at least twice daily. A detailed clinical exam with body weights recorded was done on the day of dosing (day 0).

5. Ocular observations: At 1, 24, 48, and 72 hours after instillation of the test material, test animal eyes were examined for signs of irritation with the aid of an auxiliary light source and scored according to an ocular grading system based on Draize. At the24-hour examination, all test and control eyes were subjected to fluorescein staining, and any residual test substance was gently rinsed from the eye using physiological saline. If uptake of fluorescein stain was noted at 24 hours, a fluorescein exam was repeated on the affected eye at each subsequent interval until a negative response was obtained.

II. RESULTS

A. MORTALITY: All rabbits survived the study.

B. OCULAR OBSERVATIONS: Observations are summarized in Table 1. There were no observations of corneal opacity, iritis, discharge, or conjunctival erythema or chemosis in any treated eye at any time during the study, and the treated eyes did not show fluorescein uptake at the 24-hour observation. [One control eye showed fluorescein dye retention and a superficial mechanical abrasion to the cornea at the 24-hour observation, and both findings resolved prior to the 48-hour observation.] Mean Total Scores were 0.00 at all time points. A Maximum Mean Total Score of 0.00 classifies the test material as "non-irritating" according to the Kay and Calandra scoring system.

Table 1.	Number "positive"/Number treated					
	Hours					
Observations	**1**	**24**	**48**	**72**	**168**	**336**
Corneal Opacity	0/3	0/3	0/3	0/3	0/3	0/3
Iritis	0/3	0/3	0/3	0/3	0/3	0/3
Conjunctivae:						
Redness*	0/3	0/3	0/3	0/3	0/3	0/3
Chemosis*	0/3	0/3	0/3	0/3	0/3	0/3
Discharge*	0/3	0/3	0/3	0/3	0/3	0/3

* Score of 2 or more required to be considered "positive".

III. CONCLUSION

STUDY AUTHOR'S CONCLUSIONS: According to the Kay and Calandra Evaluation Criteria, OPC-721 is considered to be a non-irritant to the ocular tissue of the rabbit and would be assigned to EPA Toxicity Category IV for labelling.

DEFICIENCIES: Clinical signs other than ocular observations were not reported and are assumed negative.

IV. REFERENCES:

Draize, J.H., G. Woodard and H.O. Calvery. 1944. Journal of Pharmacology and Experimental Therapeutics. 82: 377-390.

Kay, J.H. and J.C. Calandra. 1962. Interpretation of Eye Irritation Tests. Journal of the Society of Cosmetic Chemists. 13: 281-289.

National Research Council. 2011. Guide for the Care and Use of Laboratory Animals (8th ed.). Washington, DC. The National Academies Press.

APPENDIX I: Description of Ocular Reactions

Scale for Scoring Ocular Lesions

Cornea

A. Opacity-degree of density (area most dense taken for reading)

No opacity	0
Slight dulling of normal luster	+
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Nacreous areas, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through the opacity	4*

B. Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Score = AxBx5 Total Maximum Score = 80

Iris

A. Grades

Normal	0
Marked deepened rugae, congestion, swelling, moderate circumcorneal hyperemia or injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these).	2*

Score = Ax5 Total Maximum Score = 10

Conjunctive

A. Redness: (refers to palpebral and bulbar conjunctive excluding cornea and iris)

Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	2*
Diffuse beefy red	3*

B. Chemosis: lids and/or nictitating membranes

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

C. Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score = (A + B + C)x2 Total Maximum Score = 20

* Reaction indicates a positive effect

DATA EVALUATION RECORD

Review by: Donna L. Fefee, D.V.M., Summitec Corporation.
EPA review by: Joel V. Gagliardi, Ph.D.

Study Type	Primary Dermal Irritation Study – Rabbit (OCSPP 870.2500).
MRID No.	501593-07.
Test Material	OPC-721 containing $1.9x10^{10}$ PFU/mL Bacteriophages active against *Xylella fastidiosa*.
Study No.	20105087.
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Charles River Laboratories, Inc., 640 N. Elizabeth Street, Spencerville, Ohio.
Title of Report	Primary dermal irritation study of OPC-721 in rabbits.
Author	K. Landin.
Study Completed	November 18, 2016.
Study Summary	In a primary dermal irritation study, three young adult male New Zealand White rabbits were dermally exposed for four hours to 0.5 mL of undiluted OPC-721 applied to 1 inch by 1 inch clipped application sites. The animals were observed at one hour after patch removal and at 24, 48, and 72 hours after patch application, and the responses were scored according to Draize. There were no observations of erythema, edema, and/or other signs of dermal irritation noted on any application site at any time point during the study. All Primary Dermal Irritation scores were 0.00 and the Primary Dermal Irritation Index was 0.00.
Classification	**ACCEPTABLE** – not dermally irritating – EPA Toxicity Category IV.
Good Laboratory Practice	Signed and dated GLP statement was provided; the study is compliant with 40 CFR part 160, except the Sponsor was responsible for the expiration date and stability and/or solubility testing of the supplied test substance.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test material:**	OPC-721.
	Description:	Transparent, colorless liquid.
	Lot/Batch #:	CPTV1-004.
	Purity:	$1.9x10^{10}$ PFU/mL.
	CAS #:	Not applicable.
	Storage conditions:	Stored in a refrigerator (5°C), protected from light.
	Microbiology:	$1.9x10^{10}$ PFU/mL.

2. **Sample preparation:** The test material was dosed as received.

3. **Controls:** None.

4.	**Test animals:**		
	Species:	Rabbit.	
	Strain:	New Zealand White; Charles River Laboratories, St. Constant, Quebec, Canada.	
	Age/weight at dosing:	3 males.	
	Source:	Approximately 13 weeks / females: 2.2-2.6 Kg.	
	Housing:	Individually in stainless-steel cages with and enrichment device (stainless-steel rattle).	
	Diet:	PMI Nutrition International Certified Rabbit Chow No. 5322; *ad libitum*. Timothy Hay Cube 3x/week.	
	Water:	Municipal water, *ad libitum*.	
	Environmental conditions:	**Temperature:**	21°C
		Humidity:	50-57%
		Air changes:	>10/hour
		Photoperiod:	12 hr dark/12 hr light
	Acclimation period:	5 days.	

STUDY DESIGN AND METHODS:

1. **In Life Dates**: Start: September 13, 2016; End: September 19, 2016.

2. **Animal assignment and treatment**: Animals were assigned to a single treated group. Treatment was sequential; following the absence of severe effects in an initial treated animal, the next two animals were treated concurrently. On Day -1, the fur was removed from the dorsal area of the trunk on each animal using a small animal clipper, taking care to avoid abrading the skin. On Day 0, 0.5 mL of the undiluted test substance was applied to a small area of intact skin and covered by a 1 inch square 4-ply gauze patch, held in contact with the skin with non-irritating tape covered by a stockinette (semi-occlusive binding) over the trunk. Following completion of exposure the coverings were removed and the site wiped with gauze moistened with distilled water. Collars were placed on each animal through Day 3.

3. **Dermal observations:** Test animals were observed daily for 3 days following exposure and also on day 7. Erythema, edema, and any other defects or irritation were recorded at 1, 24, 48, and 72 hours after patch removal and also on day 7.

4. **Clinical observations and body weights:** Cage-side observations for general condition, moribundity, and mortality were made twice daily throughout the study. The animals were weighed at the time of assignment to the study. At the time of assignment and prior to dosing on Day 0, the animals were given detailed clinical examinations.

II. RESULTS

A. MORTALITY: All rabbits survived the study.

B. DERMAL OBSERVATIONS: There were no observations of erythema, edema, and/or other signs of dermal irritation noted on any application site at any time point during the study. All Primary Dermal Irritation scores were 0.00 and the Primary Dermal Irritation Index was 0.00. According to the U.S. EPA Dermal Classification System (1988), the test material is a nonirritant.

C. CLINICAL OBSERVATIONS: There were no reported abnormalities.

III. CONCLUSION

STUDY AUTHOR'S CONCLUSIONS: Under the conditions of the test (4-hour exposure), OPC-721 is considered to be a non-irritant to the skin of the rabbit. The calculated Primary Irritation Index (PII) for the test substance was 0.00. The test substance would be assigned to EPA Toxicity Category IV for labeling.

DEFICIENCIES: Dermal responses should be scored at 24, 48, and 72 hours after patch removal rather than after application of the test material. This deficiency did not compromise the validity of the study results.

Appendix I: Description of Skin Reactions

Evaluation of Skin Reactions	Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

DATA EVALUATION RECORD

Review by: Donna L. Fefee, D.V.M., Summitec Corporation.
EPA review by: Joel V. Gagliardi, Ph.D.

Study Types	Skin Sensitization - Rabbit (OCSPP 870.2600).
MRID No.	501593-08.
Test Material	OPC-721 containing $1.9x10^{10}$ PFU/mL Bacteriophages active against *Xylella fastidiosa*.
Study No.	20105089.
Sponsor	Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-0192, Japan.
Testing Facility	Charles River Laboratories, Inc., 640 N. Elizabeth Street, Spencerville, Ohio.
Title of Report	Dermal sensitization study of OPC-721 in guinea pigs – modified Buehler design
Authors	K. Landin.
Study Completed	December 22, 2016.
Study Summary	In a skin sensitization study, ten male and ten female young adult Hartley-derived albino guinea pigs were tested with undiluted OPC-721 using the Buehler method, with a 100% concentration also used at challenge. Two separate naïve control groups of five males and five females were treated during challenge only. The study also included a positive control test of 5 animals/sex treated with α-Hexylcinnamaldehyde (HCA; 5.0% w/v in ethanol), with two additional groups of 5 animals/sex serving as naïve controls at the challenge treatments (2.5% and 1.0% w/v in acetone) or (2.5% w/v in acetone). Due to inappropriate results for the concurrent HCA positive control group, the protocol was amended to evaluate a second positive control, 1-Chloro-2, 4-dinitrobenzene (DNCB). DNCB was tested in 5 animals/sex (0.1% w/v in an ethanol/acetone mixture) with an additional group of 5 animals/sex serving as naïve controls at the challenge treatments (0.1% and 0.05% w/v in ethanol/acetone). Following challenge and subsequent re-challenge with undiluted OPC-721, no positive dermal reactions were seen in any treated or naïve control animal. For the HCA positive control, challenge at 2.5% and 1.0% w/v in acetone challenge at 2.5% w/v in acetone did not produce any "positive" irritation in any of the HCA-treated or naïve control animals; these results are consistent with the absence of sensitization, and are inappropriate for a known sensitizer. Challenge with DNCB at 0.1% w/v in ethanol/acetone resulted in "positive" dermal reactions in 10/10 DNCB-treated animals and in 9/10 naïve controls, with all positive reactions persisting from 24 hours through 48 hours; mean scores for the treated animals and naïve controls were 2.0 and 1.0, respectively. Challenge with DNCB at 0.05% w/v in ethanol/acetone resulted in "positive" dermal reactions in 10/10 DNCB-treated animals, and these persisted through 48 hours in 5/10 animals; no positive reactions were seen at either time point in naïve controls. Inappropriate results in the concurrently tested positive control animals lead to questions about the negative study results for the test material OPC-721.
Classification	**SUPPLEMENTAL** – likely not a dermal sensitizer – not a required study.
Good Laboratory Practice	Signed and dated GLP statement was provided; the study is compliant with 40 CFR part 160, except the Sponsor was responsible for the expiration date and stability and/or solubility testing of the supplied test substance.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test material:**	OPC-721.
	Description:	Transparent, colorless liquid.
	Lot/Batch #:	CPTV1-004.
	Purity:	$1.9x10^{10}$ PFU/mL.
	CAS # of TGAI:	Not applicable.
	Structure:	Stored in a refrigerator (5°C), protected from light.
	Solvent Used:	$1.9x10^{10}$ PFU/mL.

2. Sample preparation: The test material was used as received, undiluted, and, diluted in RO-DI-water.

3.	**Test animals:**		
	Species:	Guinea Pig.	
	Strain:	Hartley-derived albino; Charles River Laboratories, Stone Ridge, New York.	
	Age/weight at dosing:	43/sex (females nulliparous and non-pregnant).	
	Source:	Approximately 5-6 weeks / females: males: 328-463g; females; 327-439 g.	
	Housing:	Same-sex pairs were housed either in polycarbonate cages containing appropriate bedding or in solid bottom cages containing a PVC pipe hiding device and direct bedding material.	
	Diet:	PMI Nutrition International Certified Guinea Pig Chow No. 5026; *ad libitum*; Timothy Hay Cube 3x/week.	
	Water:	Municipal water; *ad libitum*.	
	Environmental conditions:	**Temperature:**	20-22°C
		Humidity:	43-61%
		Air changes:	>10/hour
		Photoperiod:	12 hr dark/12 hr light
	Acclimation period:	7 days.	
	Negative Control:	None.	
	Solvent (final conc'n):	RO-water in the preliminary study, and none in the main study.	

	Positive Control:	α-Hexylcinnamaldehyde (HCA) 5% in ethanol (induction) or acetone (challenges). 1-Chloro-2, 4-dinitrobenzene (DNCB) 0.1% in an ethanol/acetone mixture.

STUDY DESIGN AND METHODS:

1. In-life dates: Start: September 13, 2016; End: December 16, 2016.

2. Preliminary irritation assay: OPC-721 was evaluated in two males and two females at four application sites per animal: undiluted (100%, as received) and at 75%, 50%, and 25% concentrations in reverse-osmosis deionized (RODI) water. No dermal irritation resulted at any of these concentrations through 48 hours. The 100% (as received/undiluted) formulation was selected for induction and challenge.

3. Animal assignment and treatment: Animal assignment is shown in Table 1. One day prior to each induction, challenge, and/or rechallenge treatment, the hair was clipped from the entire left or right side of the animal (as appropriate). On each treatment day, 0.3 mL of the appropriate formulation was placed in a 25 mm Hill Top Chamber® backed by adhesive tape (occlusive patch) which was then applied to the clipped skin surface as quickly as possible. Approximately 6 hours after application, the binding materials were removed, and the test sites were wiped with gauze moistened in RODI water, followed by dry gauze, to remove any residual test or positive control substance. Inductions were done on Day 0, Day 6 (DNCB only) or Day 7 (OPC-721 and HCA), and Day 14, using sites on the animals' left side; in the case of pre-existing skin irritation, naive sites (also on the animals' left side) were used. Challenge was done on Day 28, using sites on the animals' right side; for the HCA and DNCB groups (and their respective naive controls), two sites and two separate concentrations were used at challenge. Challenge (of OPC-721 and HCA animals, only) was done on Day 35, using sites on the animals' right sides. For challenge, separate naïve groups of previously untreated animals were treated in identical manner as the test and positive control animals. It must be noted that OPC-721 and HCA were tested concurrently; the second positive control substance (DNCB) and additional test animals were added to the study due to inappropriate results for HCA.

3. Dermal observations: The application sites were examined at 24 and 48 hours after application for inductions and at 24 and48 hours after removal for the challenge, with an additional observation made at 72 hours if deemed necessary to allow further evaluation of challenge responses. Irritation was graded according to Buehler, using an alternative light source if necessary. The dermal grading system is provided in Appendix I.

4. Clinical observations and body weights: Cage-side observations for general condition, moribundity, and mortality were made twice daily throughout the study, and the animals were given detailed clinical examinations prior to dosing on Day 0. The animals were weighed on Day -1, Day 27 (the day prior to challenge dosing), and Day 34 (the day prior to challenge dosing).

II. RESULTS

A. MORTALITY: All animals survived the study.

B. DERMAL OBSERVATIONS:

OPC-721: Following the first induction, slight erythema was noted on 1/20 test animals at 24 hours and resolved prior to the 48-hour observation. There were no observations of erythema, edema, or notable dermal lesions on any treated animal following the second and third inductions, the challenge. There were no observations of erythema, edema, or notable dermal lesions on the challenge controls controls at either time point following their respective treatments.

HCA: Following the first induction, 9/10 animals had positive dermal irritation (erythema, grades 1-2) at 24 and 48 hours after application and 1/10 had slight patchy erythema (score = ±) at both time points, with very slight edema noted for 1/10 animals and focal and/or pinpoint areas of blanching (up to 10% of the test site) noted for 1/10 animals. Observations following the second induction included maximized erythema in 9/10 animals by 48 hours after application and moderate erythema in the remaining animal, with very slight to slight edema and blanching of the test site for 10/10 animals and eschar within the test site for 3/10 animals. For Induction 3, the test site was moved based on the dermal irritation noted during Induction 2. Following the third induction, moderate erythema was noted on 9/10 animals by 48 hours and slight erythema was noted on the remaining animal, with blanching within the test site on 3/10 animals, very slight edema on 5/10 animals, and skin flaking on 1/10 animals. Following challenge with 2.5% w/v HCA in acetone, an erythema score of "±"was noted for 1/10 HCA test animals at the 24-hour scoring interval (only), with no signs of dermal irritation noted on any other HCA-treated animal or naïve control at either time point. Following challenge with 1.0% w/v HCA in acetone, an erythema score of "±"was noted for 1/10 HCA test animals at the 24-hour scoring interval (only), with no signs of dermal irritation noted on any other HCA-treated animal or naïve control at either time point. Following challenge with 2.5% w/v HCA in acetone, dermal scores of "±" were noted for 1/10 HCA test animals

and 4/10 HCA challenge naive control animals at the 24-hour scoring interval. No irritation was noted on any test or naïve control animal at the 48-hour observation following challenge.

DNCB: Following all three induction exposures, all of the animals had yellow skin staining and erythema on the dose site, which ranged from slight patchy (score = ±) to moderate (score = 2), with severity generally increasing with successive inductions. Additional observations included slight edema on 8/10 test animals, blanching on 3/10 animals, and flaking skin on 3/10 test animals at one or both time points following the second and/or third inductions. Following challenge with 0.1% w/v DNCB in acetone/ethanol, moderate erythema (score of 2) was noted for all 10 DNCB test animals at both scoring intervals. Other dermal findings included yellow skin staining on all 10 animals and very slight edema for 3/10 animals. Dermal reactions in the naïve control animals included slight erythema (score of 1) on 9/10 animals at both intervals and slight patchy erythema on the remaining naïve control, with yellow skin staining on all 10 naïve control animals. Group mean dermal scores were higher in the DNCB test animals (2.0) compared to the DNCB control animals (1.0). Following challenge with 0.05% w/v DNCB in acetone/ethanol, slight erythema (score of 1) and/or moderate erythema were noted for 10/10 DNCB test animals at 24 hours and persisted through 48 hours in 5/10 animals, with yellow skin staining seen on all 10 animals and very slight edema noted for 1 male. Dermal reactions in the DNCB naïve control animals included no positive reactions; at both time points, slight patchy erythema was noted in 6/10 animals and no erythema (score of 0) was noted in4/10 animals. Group mean dermal scores were higher in the DNCB test animals (0.9) compared to the DNCB control animals (0.3).

C. CLINICAL OBSERVATIONS: Abnormal clinical signs were limited to brown or yellow fur staining of the abdominal or urogenital areas in two of the OPC-721-treated males and one male and one female from the HCA-rechallenge control group.

III. CONCLUSION

STUDY AUTHOR'S CONCLUSIONS: Based on the results of this study, OPC-721 is not considered to be a contact sensitizer in guinea pigs. The exact details are unknown, but the lack of sensitization response in the HCA-treated animals is attributed to the HCA material itself. The results of the DNCB positive control study conducted afterwards demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

DEFICIENCIES: Inappropriate results for some of the concurrent positive controls were reported.

IV. REFERENCES

NRC (National Research Council). 2011. Guide for the care and use of laboratory animals. National Academy Press, Washington D.C.

Buehler, E.V. 1965. Delayed contact hypersensitivity in the guinea pig, *Archives of Dermatology* 91:171–177.

Appendix I: Description of Skin Reactions.

OBSERVATION	DEFINITION	CODE
Erythema - Grade 0	No reaction	0
Erythema - Grade ±	Slight patchy erythema	±
Erythema - Grade 1	Slight, but confluent or moderate patchy erythema	1
Erythema - Grade 2	Moderate, confluent erythema	2
Erythema - Grade 3	Severe erythema with or without edema	3
Maximized Grade 3	Notable dermal lesions	M-3 (see below)
Edema - Grade 1	Very slight edema (barely perceptible)	ED-1
Edema - Grade 2	Slight edema (edges of area well defined by definite raising)	ED-2
Edema - Grade 3	Moderate edema (raised approximately 1 millimeter)	ED-3
Edema - Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4

NOTE: An erythema code was assigned to each test site. An edema code was assigned only if edema was present at the test site. If notable dermal lesion(s) (> Grade 1) were present, then the "Maximized Grade 3" was assigned to the test site in place of the erythema score and the type of the notable dermal lesion(s) was noted (e.g., $M\text{-}3^{ES\text{-}2}$).

Taken from page 164 (Appendix 7), MRID 501593-08.

APPENDIX 2: Notable dermal lesions.

OBSERVATION	DEFINITION/EXPLANATION	CODE
Eschar	A crust-like formation within or on the test area. Characterized as scab-like (dried blood or lymph) or dead layers of tissue/crust. The area is hardened to the touch and not very pliable. Note: Because erythema cannot be observed through eschar and eschar is considered to be a notable dermal lesion, the erythema score was maximized when eschar was present greater than ES-1. The test site was observed for reversibility in order to determine if the eschar was an in-depth injury. Coded using an area designation (see below).	--
Eschar - Grade 1	Focal and/or pinpoint areas up to 10% of test site	ES-1
Eschar - Grade 2	> 10% < 25% of test site	ES-2
Eschar - Grade 3	> 25% < 50% of test site	ES-3
Eschar - Grade 4	> 50% of test site	ES-4
Blanching	Characterized by areas of white to yellow or tannish discoloration in the test site due to a decreased blood flow to the skin. Note: An erythema score cannot be determined and blanching is considered a notable dermal lesion; therefore, the erythema score was maximized when blanching was present greater than BLA-1. The test site was observed for reversibility in order to determine if the blanching was an in-depth injury. Coded using an area designation (see below).	--
Blanching - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	BLA-1
Blanching - Grade 2	> 10% < 25% of test site	BLA-2
Blanching - Grade 3	> 25% < 50% of test site	BLA-3
Blanching - Grade 4	> 50% of test site	BLA-4
Ulceration	An open lesion in the skin possibly due to the exfoliation of necrotic tissue or eschar formation. Characterized by a crater-like area which is generally inflamed and has a moist exudate. The erythema score was maximized when ulceration was present greater than U-1. Ulceration is considered an in-depth injury. Coded using an area designation (see below).	--
Ulceration - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	U-1
Ulceration - Grade 2	> 10% < 25% of test site	U-2
Ulceration - Grade 3	> 25% < 50% of test site	U-3
Ulceration - Grade 4	> 50% of test site	U-4
Necrosis	The apparent death of a portion of tissue which may result in irreversible damage depending on the severity of injury based on the color, area and texture. It is characterized by a dark (ranging from gray to black) and often in-depth discoloration of the tissue. Because this term is considered to be diagnostic, this observation was only made with the approval of the Study Director and accompanied by a full description (the color was noted). The erythema score was maximized when necrosis was present greater than NEC-1. Necrosis is considered a notable dermal lesion and an in-depth injury. Coded using an area designation (see below).	--
Necrosis - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	NEC-1 (color)
Necrosis - Grade 2	> 10% < 25% of test site	NEC-2 (color)
Necrosis - Grade 3	> 25% < 50% of test site	NEC-3 (color)
Necrosis - Grade 4	> 50% of test site	NEC-4 (color)

Taken from pages 165-166 (Appendix 7), MRID 501593-08.

APPENDIX 3: Additional dermal observations.

OBSERVATION	DEFINITION/EXPLANATION	CODE
Desquamation or Skin Flaking	Characterized by scaling or flaking of dermal tissue with or without denuded areas. May consist of a range from dry flaking of the skin to more pronounced flaking with denuded areas (in these cases the affected area may have a slight harder "feel" to it as compared to normal tissue; however, this should not be confused with a notable dermal lesion such as eschar). Areas of eschar were not scored for desquamation/skin flaking. This finding is generally not considered significant if the test site is otherwise clear for erythema, edema, etc.	DES or SFLA
Fissuring	Characterized by cracking of the skin or eschar formation (slough and/or scab) that is associated with moist exudate. Fissuring was checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site. This observation was noted only with an ES observation. May be graded with the following criteria.	EXF
Eschar Exfoliation – Grade 1	Barely perceptible scales.	EXF-1
Eschar Exfoliation – Grade 2	Distinct scales.	EXF-2
Eschar Exfoliation – Grade 3	Pronounced flaking with denuded sites.	EXF-3
Test Site Staining or Skin Staining	Skin located at the test site appears to be stained/discolored possibly due to test substance (note color of staining).	TSS (color) or SSTA
Erythema Extends Beyond the Test Site or Skin Red	The erythema extends beyond the test site. May be referred to as "Skin Red" with an appropriate location. Note: A Study Director was contacted for erythema extending beyond the test site.	ERB or SRED
Superficial Lightening or Skin Pale	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but by itself was not considered a notable dermal lesion that resulted in a maximized dermal score. May be graded with the following criteria:	SL or SPAL
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4

Taken from page 167 (Appendix 7), MRID 501593-08.